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Original Research Article

Influence of traditional Cambodian smoking practices on the concentration of Polycyclic Aromatic Hydrocarbons (PAHs) in smoked fish processed in the Tonle Sap area, Cambodia

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ABSTRACT

Aim of this work was to in detail investigate the practices of traditionally smoke-cured fish from the Tonle Sap area, Cambodia and monitoring of the concentrations of selected polycyclic aromatic hydrocarbons (PAHs). The levels of benzo(a)pyrene (BaP), sum of 4 PAHs (ΣPAH_4) and total PAHs (ΣPAH_{12}) in 57 samples of smoked fish commonly consumed in Cambodia were determined by modified Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) – Enhanced Matrix Lipid Removal (EMR Lipid) – Dispersive Liquid-Liquid Microextraction (DMLLE) method and analysed by gas chromatography – mass spectrometry (GC/MS). The results highly exceeded the limits given by European Commission (EU) No 1881/2006. The highest ΣPAH_4 and ΣPAH_{12} concentrations were detected in *Paralaubuca typus*, 2700 μg/kg and 16,800 μg/kg, and the lowest measured in *Paralaubuca barroni,* 76.3 μg/kg and 537 μg/kg, respectively. The results showed significant increase of ΣPAH_{12} mean values between smoking times T1 (3–16 h) and T2 (1–4 days), and when fuel wood was used. Correlation between the fat content and PAHs contamination was not observed. The high concentrations of PAHs are attributed to a combination of factors (type of fuel used and length of smoking). However, other factors cannot be excluded (fire-starting technique, temperature regulation, type of heat source).

1. Introduction

Tonle Sap in Cambodia is a fascinating water system that includes one of the largest freshwater lakes in terms of area and a river with the same name. Of the 16 million people in Cambodia, approximately 76 % (12.5 million people) live in rural areas (World Bank, 2018), and more than 66 % are economically active in agriculture. It is estimated that at least 45 % of the population works full time in fisheries or fishery-related activities (Vilain and Baran, 2016). In Cambodia, fresh fish consumption per capita was approximately 42 kg in 2007 (Ahmed et al., 1999; FAO, 2020; Hortle, 2007). Recent estimates of fresh fish and fish product consumption indicate that they represent up to 37 % and 76 % of the total and animal protein intake, respectively (FAO, 2020; Vilain and Baran, 2016). Approximately 85 % of the total fish catch comes from inland fisheries in the Tonle Sap area. Furthermore, fisheries in the Tonle Sap area are considered to be essential for the national economy and provide multiple livelihood opportunities. They directly contribute to food security in the country and represent an important source of income for a substantial part of the population (Belton and Thilsted, 2014; Vilain and Baran, 2016).

However, the availability of fish in Cambodia depends on the season due to the monsoon period and subsequent changes in the water levels in Tonle Sap Lake. With its high content of water, fish is a highly perishable material. Therefore, fast, and basic processing and subsequent preservation are crucial to ensure a continuous supply of protein throughout the year.

Smoking is one of the oldest food preservation techniques, dating back to more than 9000 years ago (Essumang et al., 2013; Kartalovic et al., 2015; Simko, 2002), and it is still widely used in the food industry. It is estimated that this technology is used to treat 40–60% of meat products and 15 % of fish (Stołyhwo and Sikorski, 2005) worldwide. Smoked fish products are favoured for their longer shelf life compared to fresh unprocessed fish, their lightweight and specific organoleptic properties. Moreover, smoked fish contains the second highest amount

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of protein with the second highest preservation effect after dried fish products (Hortle, 2007). However, smoked products can also be sources of contaminants formed during the process itself (Basak and Kim, 2015). The rate of deposition and absorption of various components depends on the temperature, humidity, flow rate, density of the smoke, water solubility and volatility of particular compounds (Stołyhwo and Sikorski, 2005). The most well-known xenobiotics formed during smoking are polycyclic aromatic hydrocarbons (PAHs) (Bansal and Kim, 2015). This large group of organic compounds is characterized by structures composed of two or more aromatic rings, lipophilicity, and relatively high stability in the environment (Pensado et al., 2005; Bansal and Kim, 2015; da Silva et al., 2017). The most abundant PAHs are classified by the International Agency for Research on Cancer (IARC, 2012, 2010, 1987) as probably carcinogenic (group 2A) (benzo(a)pyrene) or possibly carcinogenic (group 2B) (e.g., chrysene, indeno[1,2,3c,d]pyrene, etc.). Despite their high abundance in the environment the main exposure route to humans is via food (Rengarajan et al., 2015; Xia et al., 2010). Nevertheless, their contents in high-risk food groups (such as smoked, grilled, or baked products) can be minimized by good manufacturing practices during food processing.

Currently, commercial smoking is performed by modern controlled methods that effectively eliminate the incidence of PAHs in the final products. However, traditional methods of smoking in smokehouses (kilns) are still popular and very common in households or small-scale production. However, smoking under uncontrolled technological conditions and nonexistent legislative measures lead to enormous PAHs contents in smoked foods (Šimko, 2005). Consequently, these products can be associated with potential health hazards (Alomirah et al., 2011; Stołyhwo and Sikorski, 2005). A typical example might be traditional Khmer smoked fish. Smoking fish in traditional kilns in Cambodia typically involves treating presalted or sundried whole, eviscerated or filleted fish with smoke. The smoke is generated in direct contact with the product by smouldering wood and shavings or charcoal.

Preliminary studies conducted in the Tonle Sap area showed that the MLs (maximum limits) of the studied samples greatly exceeded the limits for both benzo(a)pyrene (BaP) and the sum of four PAHs (ΣPAH_4) given by European Commission (EC) No 1881/2006 (Slámová et al., 2017). First, the high contents of PAHs can be caused by insufficiently controlling the temperature during smoking due to the lack of an effective temperature regulation system. Moreover, local people have little knowledge of controlling the smoking temperature to meet quality standards. Second, incorrect practices are implemented during fire initiation. It is common practice to use garbage, plastics, or gas to start fires faster.

Considering that smoked fish is a regular part of the diet of the Cambodian population, we assume that these products can play a significant role in the total burden of PAHs for Cambodians and thus can contribute to a higher incidence of cancer. Nevertheless, there is currently a lack of studies of the traditional smoking process and PAHs occurrence in fish products in Cambodia. Therefore, the aim of this study was to investigate in detail the traditional practices for smoke-cured fish in the Tonle Sap area and their influence on the final contents of selected PAHs in smoked fish. This research, which involved a higher number of samples (57 samples of 18 fish species), might provide a more complex view of this problem.

2. Materials and methods

2.1. Sampling and questionnaire survey

In total, 63 samples of 18 species of smoked fish were collected (Table 1) directly from the smokehouses of 30 producers. Fish samples were collected from 6 villages (Spean Trong, Kandal, Phsar Leur, Preak Trab, Chamkar Reusey village and Lor Eit) in 3 provinces (Kampong Cham, Kamong Chhnang and Battambang) in the wetlands of the Tonle Sap Lake area in Cambodia (Fig. 1). Samples were collected during the period from October to December 2018. The samples were frozen (-20 °C) until analyses were performed. The fish samples were determined to be species of the order Siluriformes, Osteoglossiformes and Cypriniformes and the family Siluridae, Notopteridae, Clariidae, Cyprinidae, Pangasiidae and Belonidae. To gather supplementary data to evaluate the final PAHs concentrations in the fish samples, a questionnaire survey was conducted. This survey was given to all the producers (i.e., 30) involved in the sample collection (Fig. 1). To better understand the whole process of traditional smoking, three groups of questions were prepared: the introductory part was related to the producer and the source of the raw material, the technical part focused on parameters affecting the deposition of PAHs, and marketing and selling practices were also surveyed. All the data were collected in local units and names, and all the interviews and questionnaires were conducted in the Khmer language and then translated to English. Additionally, temperature data were collected for products for which the production was ongoing. For that purpose, a one-channel Testo 925 thermometer (Testo s.r.o., Prague, Czech Republic) with a Testo TE type K immersion probe (Testo s.r.o., Prague, Czech Republic) was used.

2.2. Determination of PAHs in smoked fish

2.2.1. Chemicals and reagents

A standard mixture of 16 important polycyclic aromatic

Table 1
Fish species collected for sampling listed according to Family and Order.

Scientific name	Family	Order	Reference	No. of samples collected
Xenentodon cancila	Belonidae	Beloniformes	*Hamilton, 1822*	1
Clarias *spp.*	Clariidae	Siluriformes	*Linnaeus, 1758*	6
Henicorhynchus siamensis	Cyprinidae	Cypriniformes	*Sauvage, 1881*	10
Hypsibarbus malcolmi	Cyprinidae	Cypriniformes	*Smith, 1945*	1
Labeo chrysophekadion	Cyprinidae	Cypriniformes	*Bleeker, 1849*	2
Osteochilus schlegeli	Cyprinidae	Cypriniformes	*Bleeker, 1851*	1
Paralaubuca barroni	Cyprinidae	Cypriniformes	*Fowler, 1934*	3
Paralaubuca typus	Cyprinidae	Cypriniformes	*Bleeker, 1864*	3
Puntioplites proctozystron	Cyprinidae	Cypriniformes	*Bleeker, 1865*	1
Rasbora hobelmani	Cyprinidae	Cypriniformes	*Kottelat, 1984*	7
Notopterus notopterus	Notopteridae	Osteoglossiformes	*Pallas, 1769*	1
Belodontichthys truncatus	Siluridae	Siluriformes	*Kottelat & Ng, 1999*	2
Micronema hexapterus	Siluridae	Siluriformes	*Bleeker, 1851*	6
Ompok bimaculatus	Siluridae	Siluriformes	*Bloch, 1794*	4
Pangasius elongatus	Siluridae	Siluriformes	*Pouyaud, Gustiano & Teugels, 2002*	1
Phalacronotus bleekeri	Siluridae	Siluriformes	*Günther, 1864*	4
Phalacronotus micronemus	Siluridae	Siluriformes	*Bleeker, 1846*	2
Wallago attu	Siluridae	Siluriformes	*Bloch & Schneider, 1801*	2
Total of fish samples				**57**

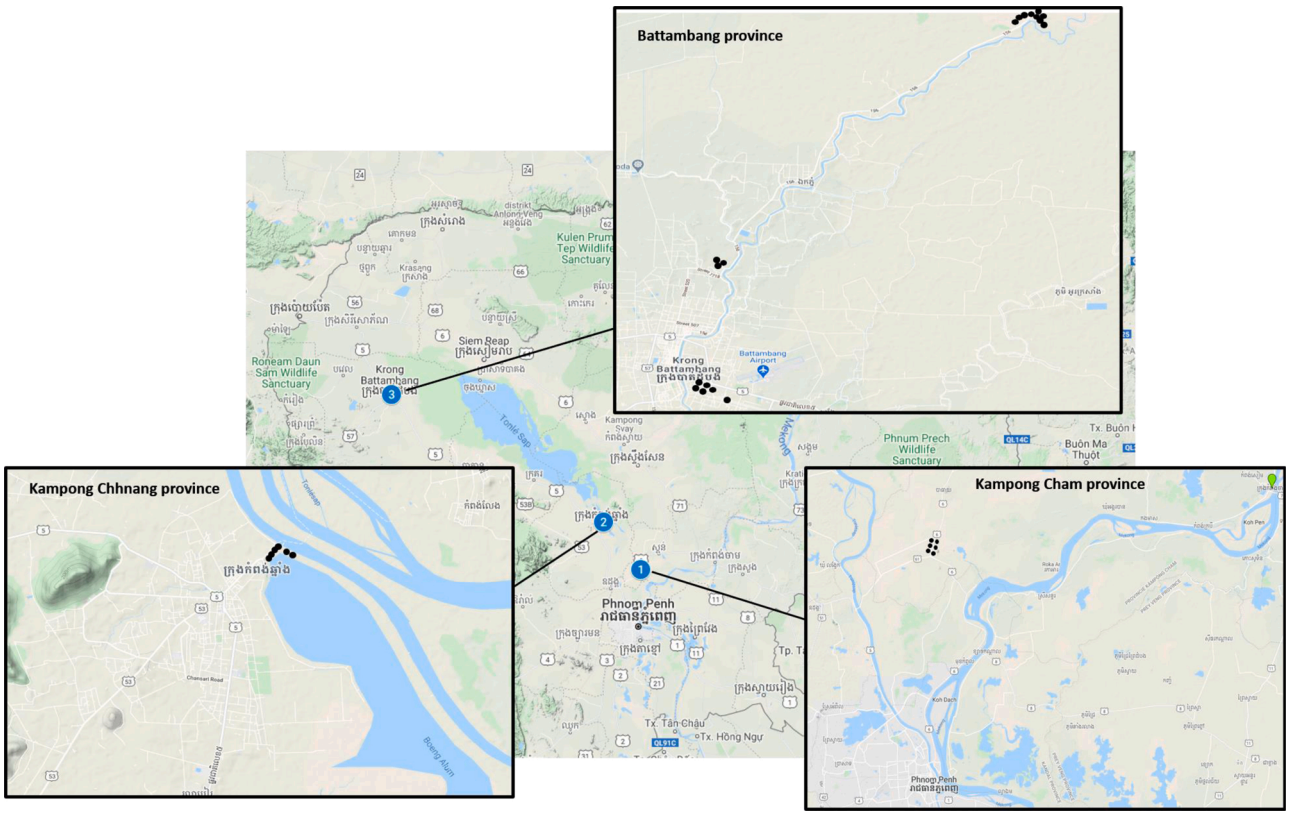

Fig. 1. Location of smoke-cured fish producers all provinces, Tonle Sap area, Cambodia.

hydrocarbons (QTM PAH mix) and a mixture of deuterated internal standards (Semivolatile Internal Standard Mix) were purchased from Sigma Aldrich, Prague, CZ. Anhydrous magnesium sulfate (p.a. anhydrous, ReagentPlus®, ≥99.5 %) and sodium chloride (p.a. ReagentPlus®, ≥99.5 %) were purchased from Sigma-Aldrich, Prague, CZ. The material EMR-Lipid (Enhanced Matrix Removal – Lipid) was obtained from Agilent Technologies, Santa Clara, USA. Acetonitrile, chloroform, and hexane were purchased from VWR Chemicals, Prague, CZ. Stock, intermediate and working standard solutions of PAHs and the internal standards were prepared in hexane. Calibration standards of PAHs with concentrations ranging from 2 to 2500 ng/mL were prepared by diluting the standard mixture solution to the corresponding hexane volume.

2.2.2. Sample preparation for GC/MS analysis

First, the fish samples were homogenized using a laboratory blender (IKA, Staufen, DE) and liquid nitrogen. The PAHs were extracted by the QuEChERS – EMR Lipid – DMLLE method described by Slámová et al. (2020). Briefly, 1 g of dried smoked fish was weighed into a 50 mL Falcon tube and spiked with an internal standard solution at a level of 500 $\mu g.kg^{-1}$, and then 10 mL of acetonitrile and 5 mL of ultrapure water from PURELAB flex 1 (Elga LabWater Veolia, High Wycombe, UK) were added. The tube was vigorously hand-shaken for 2 min and then allowed to stand for 10 min to properly rehydrate the dried samples. Next, 5 g of magnesium sulfate and 1.5 g of sodium chloride were added, and the tube was vigorously hand-shaken for another 2 min. The sample was then centrifuged at 4600 rpm and 0 °C for 15 min. The supernatant (7 mL) was transferred to 15 mL tubes containing 1 g of the EMR-Lipid sorbent previously activated with 5 mL of ultrapure water. After the addition of the supernatant, the tube content was vortexed for another 1 min. This mixture was then centrifuged at 4600 rpm and 0 °C for 15 min. Five millilitres of the obtained supernatant was transferred to a 15 mL tube containing 1.6 g of magnesium sulfate and 0.4 g of sodium chloride, and the tube was shaken for 2 min. Then, the sample was centrifuged at 4600 rpm and 0 °C for 15 min. The upper layer (2 mL) was transferred to a 15 mL tube containing 6 mL ultrapure water and 200 µL chloroform, the tube was shaken for 2 min, and the mixture was allowed to stabilize. The bottom chloroform layer was transferred to a vial and allowed to evaporate until dry. Finally, the evaporated sample was diluted in 500 µL of hexane and analysed by gas chromatography–mass spectrometry (GC/MS). All samples were prepared in triplicate.

2.2.3. Instrumentation

Analyses were conducted on a GC 7890A instrument coupled to a 5975C MS quadrupole detector (Agilent Technologies, Santa Clara, California, USA). The samples were separated using a VF5-ms column (30 m ×0.25 mm ×0.25 µm; Agilent Technologies, Santa Clara, California, USA) under a constant He flow (1 $mL.min^{-1}$). The GC oven was operated according to the following temperature program: initial temperature: 50 °C (1 min), 15 °C/min to 150 °C, 8 °C/min to 310 °C, and 310 °C (10 min). The sample (1.0 µL) was injected in splitless mode at 280 °C, the MS instrument was operated in the internal ionization mode, and scans were performed from *m/z* 45–500 in full scan mode to evaluate the quality of the sample extracts. To quantitatively analyse the PAHs ion monitoring mode (SIM mode), quantitative ions were used. The temperatures of the transfer line, ion source, and quadrupole were set to 280, 230 and 150 °C, respectively.

2.2.4. PAHs identification and quantification

Data acquisition and processing were performed using Agilent software (Agilent MSD ChemStation E.02.01.1177) and the NIST 2.0 library (National Institute of Standards and Technology, Gaithersburg, Maryland, USA). The PAHs were identified by comparing the retention times of the peaks and target ions with those obtained from a standard mixture of PAHs. The quantification was performed by internal standard calibration using the standard solutions of each of the PAHs and corresponding IS (phenantrene-d10, chrysene-d12, perylene-d12). Altogether, 12 PAHs, namely, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[a]anthracene, benzo[b]

fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo[a,h] anthracene and benzo[ghi]perylene, were identified.

2.2.5. Method validation

The validation of the extraction method is in detail described in Slamova et al. 2019. In this experiment the validation was performed as follows. The reagent blank and matrix blank represented by defatted fish sample mixed with commercial fish oil free from PAH (Mollers, Leknes, Norway) were used to evaluate possible background level of PAH. The matrix blank spiked at two levels 50 and 500 ng.g^{-1} of PAH was used to evaluate accuracy of the method. The recovery values of PAHs spiked samples were in acceptable ratio of 50%–120% established by Commission Regulation 836/2011 (EC, 2011) and ranged between 95 to 120 % and 68 to 110 % for 50 and 500 ng.g^{-1}, respectively. The average recovery values for PAHs are given in Table 2. Linearity of calibration range from 5−2000 ng.ml^{-1} was for all PAHs >0.99: LOD and LOQ for each analyte were expressed as 3 x sb/m and 10 x sb/m, where sb is standard deviation of blank and m is slope of calibration curve (see Table 2). Again, all presented values fall within the criteria established by the Commission Regulation 836/2011 (EC, 2011). Slightly higher LOQ values were determined for benzo[b]fluoranthene and benzo[ghi] perylene, considering the degree of contamination of fish by PAHs the LOQ values still fit for purpose. Total ion chromatograms together with EIC chromatograms of spiked and real samples are shown at Fig. 2. Spectra of benz(a)pyrene and benzo[b]fluoranthene in TIC and EIC are shown in Fig. 3.

2.2.6. Determination of the fat content in fish by a modified Van Handel method

Due the limited amount of sample, the total content of fat was determined by the microquantity colorimetric sulfo-phospho-vanillin method described by Anschau et al. (2017) with slight modifications. Homogenized fish samples (40–100 mg) were extracted for 5 min in a 4 mL chloroform:methanol mixture (1:1). Then, 1 mL of a 0.9 % NaCl solution in water was added, and the tube was vortexed for 30 s. Falcon tubes containing the homogenate were centrifuged for 5 min at 9000 rpm to separate the chloroform layer with fat from the rest of the sample. Aliquots of the chloroform layer (250 µL) were transferred to glass tubes. Meanwhile, a six-point calibration (1–40 mg.mL^{-1}) was prepared from commercially available fish oil (Moller's) in acetone, and aliquots (250 µL) were transferred to glass tubes. The tubes with the samples and calibration standards were placed in a dry heat block at 100 °C until the solvent was evaporated (approx. 10 min). After the tubes cooled, 250 µL of concentrated sulfuric acid was added, and the sample was again heated for 10 min in a dry heat block (100 °C). Finally, 2.25 mL of the phospho-vanillin reagent (300 mg vanillin, 50 mL hot distilled water and 200 mL of 85 % o-phosphoric acid) was added to the cooled sample and properly mixed. After 5 min, 100 µL of the samples and calibration standards was transferred to a 96-well microtiter plate, and the absorbance was measured on a Biotek reader (SYNERGY H1, USA) at a wavelength of 490 nm. All samples were prepared in triplicate, and three technical replications of the measurements were performed. The results are expressed as the percentage of fat in dry fish. All chemicals were analytical grade and delivered by VWR (Czech Republic).

Table 2
Method validation average recovery, linearity, LOD (limits of detection) and LOQ (limits of quantification).

	linearity	LOD	LOQ	average recovery (%)		
PAH		µg.kg-1	µg.kg-1	average		SD
Fluorene	0.998	0.06	0.63	85.6	±	2.9
Phenantrene	0.999	0.03	0.34	81.2	±	4.8
Anthracene	0.998	0.04	0.38	87.4	±	5
Fluoranthene	0.997	0.07	0.74	84	±	4.1
Pyrene	0.998	0.09	0.87	78.8	±	4.5
Chrysene	0.991	0.09	0.93	110	±	6.5
Benz[a]anthracene	0.991	0.09	0.94	81	±	5.3
Benzo[b]fluoranthene	0.998	0.16	I.61	74.5	±	4.2
Benzo(a)pyrene	0.996	0.09	0.86	87.5	±	14
Indeno[1.2.3-cd]pyrene	0.993	0.19	0.72	58.2	±	15
Dibenz[a.h]anthracene	0.995	0.09	0.94	85.5	±	15
Benzo[ghi]perylene	0.99	0.17	1.7	67.6	±	14

2.2.7. Statistical analysis of the data

The data obtained from the laboratory measurements were processed in Microsoft Office 365 Excel and then statistically analyzed using the STATISTICA 12 software. Analysis of variance (ANOVA) was applied to the PAHs concentration levels with a significance level of $\alpha = 0.05$. For descriptive statistics, a value of zero was assigned to PAHs concentrations below the LOD. Correlations were used to assess the relationship between the PAHs concentration and total fat content.

3. Results and discussion

3.1. Questionnaire survey data for traditionally smoke-cured fish in the Tonle Sap area

Based on a questionnaire survey, we can briefly describe the traditional production of smoke-cured fish in the Tonle Sap area. In this study, the small-scale producers could be divided into three groups with reported daily production DP1 (400−100 kg), DP2 (100–500 kg) and DP3 (500–1000 kg), which accounted for 37 %, 23 % and 33 %, respectively. Although the production places as well as households are often next to the water source, more than 60 % of producers buy raw products from local fisherman or on the market. The producers identified 18 fish species mainly processed by smoking. The variability of fish species is affected by the season and financial capacity of the producer. Generally, traditional smoking kilns are simple wooden or brick structures with various levels of ventilation and numbers of smoking trays (1–4). All of them use direct smoking with a direct source of heat. Selected parameters and responses about traditional smoking are shown in Table 3. More than 70 % of respondents reported using wood as fuel. It is common to use mixtures of locally available wood; however, 50 % and 20 % of respondents reported that most collected and used wood were from *Barringtonia asiatica* and *Hevea brasiliensis*, respectively. Other types of fuel reported included charcoal (13 %) and a combination of both charcoal and wood (10 %). The distance between the heat source and the first level of smoking trays varied between 50 and 100 cm. Although the temperature was not measured at all production sites, approximately 23 % of the measured values for the first tray (next to the product) varied between 80 and 100 °C. The time of smoking was reported to be up to 16 h (T1), 1–4 days (T2), one week (T3) and up to 10 days (T4) by 40 %, 47 %, 10 % and 3% of the respondents, respectively. The fire starters, which are important factors in PAHs generation, mainly included the following techniques: preparation of netlike structures for better circulation of air (40 %), plastic bags (>26 % respondents) and sawdust (23 %), and other reported fire starters were gas, coconut peel and palm oil seeds. More than 50 % of respondents also used materials such as paper cartons (69 %), metal sheets (13 %), grass mats (6%) or plastic rice bags (12 %) to cover the product during the process of smoking, mainly when smoking kilns with open structures were employed. This practice might cause additional contamination as glues and other substances are released from the covering material and burned. Indeed, practices such as burning any kind of waste to produce smoke can lead to increased concentrations of PAHs (Codex Alimentarius, 2009; Ledesma et al., 2016). Another aspect affecting PAHs deposition and accumulation is the use of packaging and storage and selling practices. More than 44 % respondents stored their products hanging outside of the smokehouse; some stored them directly on smoking trays inside the smokehouse (less than 20 %) or in the smokehouse itself (28 %), where the continuous production and subsequent deposition of PAHs might occur; and products were also stored in

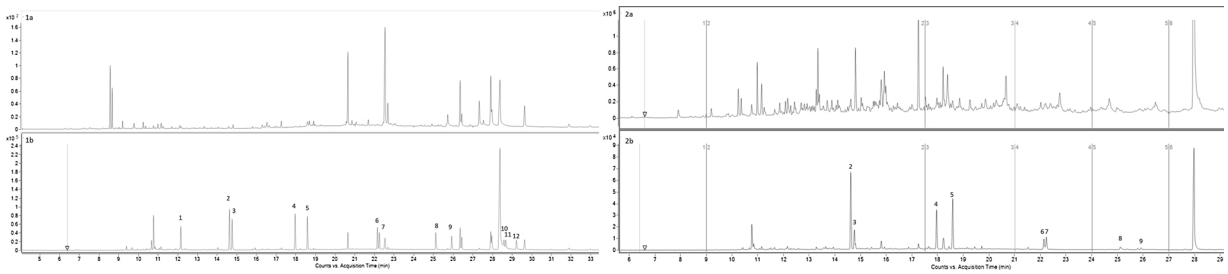

Fig. 2. Example chromatograms of fish samples, 1a – TIC of fish spiked sample, 1b – EIC of fish spiked sample, 2a TIC of real fish sample, 2b – EIC of real fish sample, 1 Fluorene, 2 Phenantrene, 3 Anthracene, 4 Fluoranthene, 5 Pyrene, 6 Benz(a)anthracene, 7 Chrysene, 8, Benzo[b]fluoranthene, 9 Benzo(a)pyrene, 10) Indeno[1,2,3-cd]pyrene, 11, Dibenz[a,h]anthracene, 12.Dibenz[a,h]anthracene.

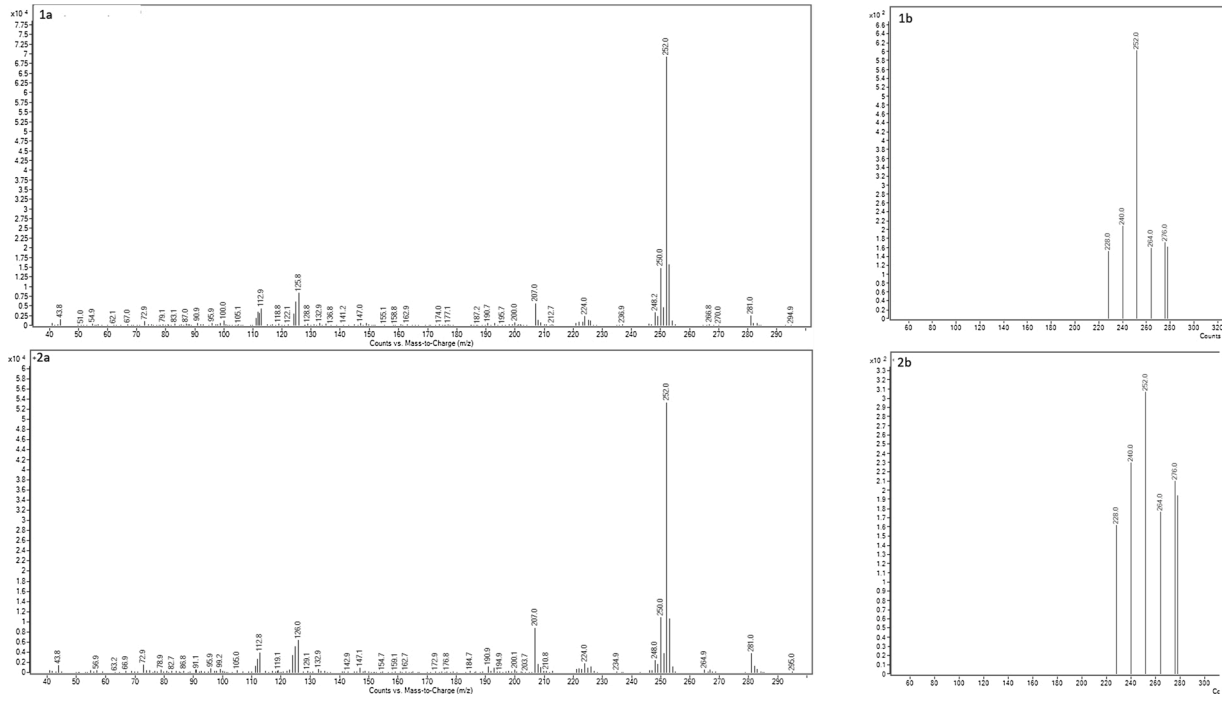

Fig. 3. Mass spectra of benz[a]pyrene and benzo[b]fluoranthene, 1a mass spectra of benzo[b]fluoranthene in scan mode, 1b mass spectra of benzo[b]fluoranthene in SIM mode, 2a mass spectra of benz[a]pyrene in scan mode, 2b mass spectra of benz[a]pyrene in SIM mode.

paper boxes where contamination from insects or rodents is common.

3.2. Concentrations of PAHs in smoked fish in the Tonle Sap area, Cambodia

3.2.1. PAHs concentrations in traditionally smoked fish products in Cambodia

In 2008, the European Food Safety Authority (EFSA) declared that by itself, BaP (benzo[α]pyrene) is not an appropriate marker for the occurrence of PAHs in food. Therefore, a combination of four specific PAHs, ΣPAH_4 (benzo[α]pyrene, chrysene, benzo[α]anthracene, benzo[β]fluoranthene), was introduced as a more accurate marker (Bansal and Kim, 2015; EFSA, 2008). In all the studied smoked fish samples, the results for PAH_4 (ΣPAH_4) and the total PAHs (ΣPAH_{12}) were expressed as the mean and standard deviation in μg PAHs per kg of dry fish matter. In total, n = 57 for the 18 fish species that were tested. The levels of BaP, ΣPAH_4 and ΣPAH_{12} found in the samples of traditionally smoked fish from the Tonle Sap area, Cambodia, are shown in Table 4. In general, the concentrations of PAHs highly exceeded the limits recommended by EU regulation (European commission (EC), 2011). Considering the fact that several studies have reported a very low PAHs concentrations or concentrations near the detection limits in fresh fish samples (Asamoah et al., 2021; Duedahl-Olesen et al., 2010; Le Dû-Lacoste et al., 2013; Stołyhwo and Sikorski, 2005; van der Oost et al., 2016), because of their ability to metabolize PAHs into water-soluble derivatives (Santana et al., 2018) and due to the low concentartions of sum of 26 PAHs, 18 PAHs and 14 PAHs equal to 51.5 ng/g, 47.0 ng/g and 39.5 ng/g, respectively in the sediments from Tonlé Sap lake reported by the study of Saha et al. (2009). We can suppose that smoking was the major contributor to the high PAHs levels in analyzed fish samples. The highest contents of ΣPAH_4 and ΣPAH_{12} were determined to be 2700 μg/kg and 16,800 μg/kg, respectively, in *Paralaubuca typus* (Producer 7), followed by 3780 μg/kg and 13,500 μg/kg, respectively, in *Labeo chrysophekadion* (Producer 2). Interestingly, *Paralaubuca barroni* from the same producer had the lowest measured mean values of ΣPAH_4 and ΣPAH_{12} (76.3 μg/kg and 537 μg/kg, respectively). Fasano et al. (2015) reported comparably high total PAHs contents in traditionally smoked sausage; they ranged from 313 to 3480 μg/kg, with an average value of 1780 μg/kg when the whole product (meat and casing) was taken into account. Several authors (Basak et al., 2010; Ciecierska and Obiedzinski, 2007; Ledesma et al., 2014) found that skin serves as a barrier to PAHs in smoke. However, in this study, we used whole fish samples because smoke-cured fish products are consumed with the skin in Cambodia. This could also explain the higher total mean values compared with those of other studied

Table 3
Selected responses to parameters of traditional smoking.

Question	Responses	% of respondents	Question	Responses	% of respondents
Fuel	Wood	77 %	**Fire-starting techniques**	Coconut peel	3%
	Charcoal	13 %		Sawdust	23 %
	Both	10 %		Palm oil seeds	3%
Fuelwood species	*Havea brasiliensis*	20 %		Plastic bags	26 %
	Combertum trifoliatum	10 %		Net structure - 0.5 m distance	40 %
	Barringtonia asiatica	50 %		Gas	3%
	Cashew tree	3%	**Frequency of smoking**	Daily	63 %
	Barringtonia acutangula	7%		2–3 times a week	20 %
	Mallotus anisopodus	7%		Weekly	10 %
	Tamarindus indica	3%		Other - dependent on the raw material	7%
Distance from fire	< 50 cm	3%	**Storage**	At smoke house	28 %
	< 60 cm	23 %		At smoking trays	17 %
	< 70 cm	33 %		Hanging	44 %
	> 70 cm	33 %		In boxes	11 %
	N/A	7%	**Source of fish**	Fishing	40 %
Temperature	40–80 °C	17 %		Buying	60 %
	80 -100 °C	23 %	**Daily production**	1–30 kg	37 %
	> 100 °C	7%		40–100 kg	23 %
	N/A*	53 %		100–500kg	33 %
Length of smoking	3–16 h**	40 %		500–1000 kg	7%
	1–4 days	47 %	**Selling practices**	Personally, on the local market	26 %
	7 days	10 %		Customers coming individually to your house	42 %
	Up to 10 days	3.0 %		Through the middleman	29 %
Use of additional technique	Carton	69 %		Directly to some bigger company or supermarket	3%
	Grass mat	6%			
	Plastic Rice bag	12 %			
	Metal sheet	13 %			

* N/A - data not available.
** h – hours.

products. In the same study by Fasano et al. (2015), smoked paprika was analysed in addition to traditional sausage. The results were significantly higher than those obtained for the sausage, and in principle, the technology used, and the results were more similar to those of the fish samples in this study. The paprika was smoked by direct smoking with smoke produced from oak wood for 10–15 days, and it had no casings to protect the final product from the smoke and PAHs. For chorizo sausage, the results for BaP, ΣPAH_4 and ΣPAH_8 ranged from 3.1, 38 and 41 μg/kg to 98, 1370 and 1510 μg/kg, respectively, and for paprika, the minimal and maximal values of PAH_4 (ΣPAH_8) were 593 μg/kg (639 μg/kg) and 3200 μg/kg (3480 μg/kg), respectively. Similarly, the high concentrations reported by Fasano et al. (2015) and in this study are also consistent with the results of Ciecierska and Obiedzinski (2007) and Šimko (2005). The traditional method of smoking leads to higher PAHs contamination than industrial processes. Various studies also pointed out that compared with other meat and nonmeat foodstuffs, fish accumulates the most PAHs (Singh et al., 2016). This is because PAHs are lipophilic in nature and fish contains higher amounts of fat than other foodstuffs. Xia et al. (2010) published results for 25 food samples that indicated that fish contained the 3rd highest concentration of PAHs, 160 μg/kg (wet weight). Another study reported that in general, the PAHs content in fish samples was considerably higher than that in smoked meat (EFSA, 2008; Plaza-Bolaños et al., 2010). Smoked fish from a Nigerian fishing settlement was found to have the highest concentration of BaP in fish (6780 μg/kg) (Anyakora et al., 2005). Although the contamination of fish by PAHs from water has also been discussed, studies have confirmed that smoked and charbroiled/grilled products contain more PAHs than their uncooked counterparts (Rengarjan, 2015). The highest and lowest mean values from the same producer might have resulted from collecting samples on different smoking days to obtain a higher diversity of collected fish species. The observed differences in the PAHs levels could also be a function of the fish fat content. Very few studies have investigated the PAHs contents of smoked foods commonly consumed in Southeast Asian countries, especially in Cambodia, even though the results are alarming and indicate contamination levels comparable to those in industrial and heavily populated areas.

Table 5 shows the mean PAHs concentrations in one fish species. The highest mean values of ΣPAH_4 and ΣPAH_{12} were measured in samples of *Paralaubuca typus* (1640 μg/kg and 9170 μg/kg, respectively), followed by samples of *Labeo chrysophekadion* (2150 μg/kg and 8410 μg/kg), respectively. Samples of *Wallago attu* had the lowest mean ΣPAH_4 and ΣPAH_{12} concentrations of 180 μg/kg and 1100 μg/kg, respectively. The content of PAHs within one species varied greatly. This trend can be mainly explained by differences in, e.g., age, season of catch or diet within each species (Rasoarahona et al., 2005). In addition to the fat content and species, it was reported that the size of the fish/sample might affect the level of contamination. In one study, Hokkanen et al. (2018) observed that small fish samples contained higher median PAH levels than larger fish samples. Additionally, PAHs content variations were attributed to nonhomogenous smoke dispersion in traditional ovens (Basak et al., 2010).

3.2.2. *Effect of the length of smoking on PAHs formation and concentration*

One of the parameters known to affect the level of contamination by PAHs is the length of the smoking process, because of the exposure to smoke and subsequent pyrolysis of fat drippings from the product (Essumang et al., 2012; Viegas et al., 2012). A longer smoking time is known to improve the shelf life of fish by significantly reducing the moisture and lipid contents, which would otherwise cause rancidification and spoilage of smoke-cured fish (Essumang et al., 2013). Because traditional smoking kilns use direct smoking and the trays have a large-diameter mesh, fat pyrolysis is highly likely to increase the PAHs concentration. On the other hand, all the producers claimed to rotate the product on the smoking trays (if present); therefore, the distance from the fire varied depending on the stage of the smoking process. The data obtained for a given fish species from more than one producer were statistically compared to determine differences in the level of PAHs contamination due to the length of smoking. Table 4, column "Time" shows the statistically significant differences ($p < 0.05$) in the mean

Table 4
Overview of the results of mean and SD of BaP, SUM PAH4, SUM PAH12 in μg/kg for all samples, fish species (n = 3).

Producer	Fish species	Province	Fuel	Time	BaP* Mean	±SD	ΣPAH$_4$** Mean	±SD	ΣPAH$_{12}$**** Mean	±SD***
P1	*Belodontichthys truncatus*	Kampong Cham	Wood	T2	113	±2	407	±8.2	2540	±72
P3		Kampong Cham	Wood	T1	29.7	±2.3	104	±11	1190	±210
P3		Kampong Cham	Wood^{abcd}	T1^{abc}	212	±5	1080	±23	7020	±120
P6		Kampong Cham	Wood^{aefgh}	T2^{adef}	274	±11	1740	±15	9510	±180
P8		Kampong Chhnang	Wood^{bei}	T1	124	±10	707	±32	3560	±990
P9		Kampong Chhnang	Wood	T3	90.9	±12	550	±25	2620	±170
P13	*Henicorhynchus siamensis*	Battambang	Both^{cfj}	T1^{bdg}	86	±7.2	549	±61	4180	±110
P16		Battambang	Wood	T2	193	±19	1010	±190	3860	±500
P18		Battambang	Wood^{dgl}	T1^{cfh}	149	±26	1160	±76	4890	±343
P19		Battambang	Wood	T1	258	±44	2190	±110	17,200	±430
P22		Battambang	Wood^{hj}	T1	139	±4.6	1150	±300	6060	±250
P23		Battambang	Wood	T1	231	±14	1530	±200	7440	±600
P2		Kampong Cham	Both^{ab}	T2	47.3	±5.3	110	±14	690	±17
P6		Kampong Cham	Wood	T2	39	±3.7	142	±13	823	±30
P25	*Clarias batrachus*	Battambang	Charcoal^{ac}	T2	112	±0.7	408	±7.9	1980	±93
P26		Battambang	Charcoal^{bc}	T2	47	±8.8	233	±2	1400	±12
P27		Battambang	Charcoal	T2	30.4	±2	150	±15	1650	±88
P28		Battambang	Charcoal	T2	35	±0.4	121	±10	611	±41
P8	*Hypsibarbus malcolmi*	Battambang	Wood	T1	221	±22	1120	±110	4200	±440
P2	*Labeo chrysophekadion*	Kampong Cham	Both^{a}	T2	609	±78	3780	±300	13,500	±940
P6		Kampong Cham	Wood^{a}	T2	187	±42	1200	±200	6300	±940
P2		Kampong Cham	Both^{abc}	T2^{abc}	273	±8.4	1320	±60	5250	±680
P3		Kampong Cham	Wood^{adef}	T1^{adef}	57.9	±7	215	±17	1300	±220
P4	*Micronema hexapterus*	Kampong Cham	Wood^{bdgh}	T2^{bdgh}	150	±16	926	±29	4050	±200
P6		Kampong Cham	Wood^{cegi}	T2^{cegi}	123	±3.1	442	±17	1680	±160
P18		Battambang	Wood	T1	45	±1.9	314	±12	2260	±210
P23		Battambang	Wood^{fhi}	T2^{fhi}	80.1	±2.4	573	±1.8	5490	±230
P2	*Notopterus notopterus*	Kampong Cham	Both	T2	46.2	±2.6	270	±19	1340	±77
P5		Kampong Cham	Both^{ab}	T2^{ab}	38.8	±1.8	195	±10	1380	±48
P6	*Ompok bimaculatus*	Kampong Cham	Wood	T2	94.3	±17	399	±60	1100	±660
P7		Kampong Chhnang	Wood^{ac}	T1^{ac}	58.6	±5.1	364	±18	1950	±20
P11		Kampong Chhnang	Wood^{bc}	T3^{bc}	196	±28	1090	±170	3070	±340
P8	*Osteochilus schlegeli*	Kampong Chhnanhg	Wood	T1	228	±9.7	1060	±63	4730	±360
P2	*Pangasius elongatus*	Kampong Cham	Both	T2	125	±8.2	872	±81	4090	±440
P2		Kampong Cham	Both	T2	29.9	±0.6	76.3	±3.1	537	±31
P3	*Paralaubuca barroni*	Kampong Cham	Wood^{a}	T1^{a}	163	±3.1	757	±27	2820	±150
P5		Kampong Cham	Both^{a}	T2^{a}	93	±0.9	562	±4.8	3530	±87
P7		Kampong Chhnang	Wood	T1	321	±31	2700	±160	16,800	±420
P8	*Paralaubuca typus*	Kampong Chhnang	Wood	T1^{a}	300	±4.2	1380	±27	6140	±210
P14		Battambang	Wood	T2^{a}	122	±27	844	±160	4550	±440
P7		Kampong Chhnang	Wood	T1^{abc}	158	±26	750	±97	1670	±640
P10	*Phalacronotus bleekeri*	Kampong Chhnang	Wood	T4^{ad}	202	±10	1090	±54	4010	±180
P11		Kampong Chhnang	Wood	T4^{be}	241	±10	1270	±120	4480	±130
P12		Kampong Chhnang	Wood	T3^{cde}	256	±14	1140	±32	2970	±38
P1	*Phalacronotus micronemus*	Kampong Cham	Wood^{a}	T2	84.9	±29	299	±59	1370	±45
P4		Kampong Cham	Wood^{a}	T2	129	±27	707	±118	2190	±240
P8	*Puntioplites prostozystron*	Kampong Chhnang	Wood	T1	71.6	±14	415	±40	2710	±360
P13		Battambang	Both	T1	163	±48	460	±63	5080	±150
P17		Battambang	Wood	T1	326	±6.1	1770	±77	10,900	±720
P19		Battambang	Wood	T1	162	±8.4	904	±56	6570	±370
P20	*Rasbora hobelmani*	Battambang	Wood	T1	116	±5.7	823	±25	2540	±120
P21		Battambang	Wood	T1	210	±35	1200	±110	5110	±300
P23		Battambang	Wood	T1	192	±26	1090	±110	11,600	±1000
P24		Battambang	Wood	T1	125	±2	560	±24	4080	±280
P1	*Wallago attu*	Kampong Cham	Wood	T2	51.3	±9.4	155	±4.4	923	±38
P4		Kampong Cham	Wood	T2	38.9	±3.2	197	±29	1290	±59
P4	*Xenentodon cancila*	Kampong Cham	Wood	T2	91.4	±9.3	556	±2.3	2090	±300

* BaP = Benzo[*a*]pyrene.
** ΣPAH$_4$ = sum of Benzo[α]pyrene, Chrysene, Benzo[α]anthracene, Benzo[β]fluoranthene.
*** SD = Standard Deviation.
**** ΣPAH$_{12}$ = sum of Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Chrysene, Benzo[a]anthracene, Benzo[b]fluoranthene, Benzo[a]pyrene, Indeno [1,2,3-cd]pyrene, Dibenzo[a,h]anthracene and Benzo[ghi]perylene; T = smoking time: T1 (3–16 h) and T2 (1–4 days), and T3 (7 days) and T4 (up to 10 days); ^{abcdefghi} = refers to statistically significant ($p < 0.05$) difference in ΣPAH$_{12}$ concentration in dependence to used fuel or smoking time within one species.

values of BaP, ΣPAH$_4$ and ΣPAH$_{12}$ for each fish species for different processing lengths as declared by the producers. Based on these results, significant differences were observed between T1 (3–16 h) and T2 (1–4 days) for *Henicorhynchus siamensis, Micronema hexapterus, Paralaubuca barroni* and *Paralaubuca typus*, and the highest mean values of ΣPAH$_{12}$ were 9510 μg/kg (T2), 5490 μg/kg (T2), 3530 μg/kg (T2) and 6140 μg/kg (T1), respectively. In the case of *Phalacronotus bleekeri*, significant differences were found between all the declared times (T1, T3 and T4). Smoking time T4 had the highest mean values of 1270 μg/kg, 4480 μg/kg and 241 μg/kg for ΣPAH$_4$, ΣPAH$_{12}$ and BaP, respectively. For all the fish species, the highest mean value of ΣPAH$_{12}$ was measured at T1, which might be explained by differences in the generation of PAHs over time. A study by Alomirah et al. (2011) suggested that low molecular weight PAHs (LMW, containing 2–3 aromatic rings), which are more volatile than high molecular weight PAHs (HMW, containing more than 3 aromatic rings), are predominant in the smoke generated by the

Table 5
Mean BaP, PAH4 and PAH12 in μg/kg values for each fish species.

Fish species	BaP *	SD ***	PAH4 **	SD	PAH12 ****	SD
Belodontichthys truncatus	80	±46	286	±170	2000	±750
Henicorhynchus siamensis	182	±67	1180	±570	5510	±2100
Clarias batrachus	57.3	±32	183	±130	1160	±560
Hypsibarbus malcolmi	221	±22	1120	±110	4200	±440
Labeo chrysophekadion	394	±240	2150	±1600	8410	±5300
Micronema hexapterus	119	±74	592	±380	3210	±1700
Notopterus notopterus	46.2	±2.6	183	±160	1340	±77
Ompok bimaculatus	128	±81	297	±120	1270	±360
Ompok bimaculatus	66.5	±34	730	±430	2200	±900
Osteochilus schlegeli	228	±9.7	1060	±63	4730	±360
Pangasius elongatus	125	±8.2	872	±81	4090	±440
Paralaubuca barroni	105	±60	507	±310	2290	±1400
Paralaubuca typus	248	±97	1640	±840	9170	±5800
Phalacronotus bleekeri	217	±41	1090	±200	3280	±1200
Phalacronotus micronemus	99.1	±32	462	±210	1730	±420
Puntioplites prostozystron	99.2	±49	426	±360	2880	±450
Rasbora hobelmani	179	±64	933	±390	6130	±3200
Wallago attu	43.9	±8.5	180	±31	1100	±210
Xenentodon cancila	91.4	±9.3	556	±2.3	2090	±300

* BaP = Benzo[a]pyrene.
** PAH4 = Benzo[α]pyrene, Chrysene, Benzo[α]anthracene, Benzo[β]fluoranthene.
*** SD = Standard Deviation.
**** PAH12 = Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Chrysene, Benzo[a]anthracene, Benzo[b]fluoranthene, Benzo[a]pyrene, Indeno[1,2,3-cd]pyrene, Dibenzo[a,h]anthracene and.Benzo[ghi]perylene.

pyrolysis of fat drippings over the heat source and the incomplete combustion of charcoal. Based on this assumption, we can suggest that the longer the process takes, the lower the temperature is, and fewer flames are present; therefore, fewer LMW PAHs are produced (Alomirah et al., 2011). This means that the mean ΣPAH_{12}, which includes all PAHs (LMW and HMW), is higher at T1 than at longer periods (>T2). This corresponds with the results of the study of Ledesma et al. (2014), in which the BaP content increased from less than 0.24 μg/kg to 0.75 μg/kg and finally stabilized after 5 days of smoking. This trend was attributed to the fact that after 5 days, the natural pores of the casing or skin could be blocked by large tar particles in the smoke, preventing the continued penetration of PAHs. Additionally, Rose et al. (2015) reported that contrary to expectations, the concentration of PAHs decreased with time in some cases. They attributed this result to differences in the surface area and surface texture of the food and the manner in which fat was lost during cooking. Essumang et al. (2013) reported the highest mean values of all 16 PAHs to range from 250 to 1140 μg/kg at 2 h, from 595 to 1310 μg/kg at 4 h, and from 574 to 1380 μg/kg at 8 h. In contrast, Chen and Lin (1997) concluded that PAHs contamination increased with smoking time. Roseiro et al. (2012) reported that the traditionally smoked meat sausages *Painho* and *Paio tradicional* had mean values of 1400 μg/kg and 2610 μg/kg after 15 and 30 days, respectively. In this study, approximately 40 % of respondents declared the duration of smoking to be as short as 3–16 h (see Table 3). Therefore, the elevated concentrations are consistent with the abovementioned studies in which the concentrations of PAHs stabilized with time.

3.2.3. Effect of the fuel used for smoking on PAHs formation and concentration

Table 4 lists the concentration results for BaP, ΣPAH_4 and ΣPAH_{12} obtained using various fuels in the smoking process for each fish species. In Table 4, column "Fuel" shows significant differences in the PAHs concentration depending on the type of fuel used. For *Henicorhynchus siamensis*, *Labeo chrysophekadion, Micronema hexapterus* and *Ompok bimaculatus,* the highest mean values of PAHs measured when fuelwood (Wood) and a combination of fuelwood and charcoal (Both) were used were significantly different 9510 μg/kg for P6 - Wood (*Henicorhynchus siamensis*), 13,540 μg/kg for P2 - Both (*Labeo chrysophekadion*), 5490 μg/kg for P23 - Wood (*Micronema hexapterus*), and 3070 μg/kg for P11 - Wood (*Ompok bimaculatus*). A significant difference between charcoal and a combination of both fuels was only observed in the case of *Clarias batrachus*, and the highest mean value of total PAHs was 1980 μg/kg for P25 - Charcoal. In general, the chemical formation of PAHs during product smoking is due to the incomplete combustion or pyrolysis of wood (Ledesma et al., 2016). This is consistent with the results of Ross et al. (2002) and Han et al. (2020), who detected higher PAHs emissions from wood combustion than from coal combustion. In addition, more LMW PAHs were emitted in the early burning stage of wood, whereas more HMW PAHs were emitted in the later burning stage, contrary to the trend for coal. Therefore, we can suggest that a combination of both fuels leads to increased contamination by PAHs. A study by Rose et al. (2015) indicated that preparing food over charcoal can lead to elevated levels of PAHs depending on the fat content. Compared with this study, a report by Roseiro et al. (2012) also found a high level of contamination in traditional meat/blood sausages directly smoked over wood with a total PAHs content of 2290 μg/kg. They explained that the high levels of PAHs were caused by the higher temperature applied to these products at the beginning of the heat treatment. Grilling over an open fire and in direct contact with flames might result in extremely high PAHs levels, as in this study. Additionally, Garcia-Perez and Metcalf (2008) found that softwood produces more PAHs than hardwood when burned because of its high lignin content. Using this type of fuel greatly increases the PAHs in meat products. Although Table 3 shows that fuelwood, such as *Barringtonia asiatica* (mangrove) and *Havea brasiliensis* (rubber tree), which are both classified as hardwood, were mainly used for smoke curing, the results of Tekasakul et al. (2008) showed a correlation between PAHs concentrations and rubber-wood burning. However, a study from Essumang et al. (2013) noted that even if mangroves are considered to be hardwood, they might have a lower lignin content due to the malfunctioning of water-transporting tissue. Therefore, the levels of PAHs in products smoked over this wood were lower than those in products smoked over other tested fuelwoods. As shown in Table 3, the use of various fire starters was reported in this study; even gas or plastic bags were placed on wood piles to start fires. The co-combustion of plastics (polyethylene (PE) and polyethylene terephthalate (PET) with wood increased the total PAH7 (4 – 6-ring PAHs) by 43 % and 71 %, respectively, and the total PAH7 ranged from 4.5–11 mg/kg (Tomsej et al., 2018). In conclusion, co-combustion with PET resulted in a significant increase in the emissions of total PAHs. Chung et al. (2011) studied the PAHs content of meat products grilled and roasted over charcoal with gasoline for 30 min and reported a BaP content of 8.49 μg/kg. However, our measured levels of contamination were considerably higher than those reported by other authors using fire starters. Therefore, fire starter use is not the only factor affecting the level of contamination by PAHs, but it might have an important effect on the final content.

3.2.4. Effect of temperature on PAHs formation and concentration

As previously mentioned, temperature is one of the factors affecting the level of contamination by PAHs. According to a study by Ledesma et al. (2016), direct smoking can be classified as cold (15–25 °C temperature of the product) or hot (80 °C product) smoking based on the temperature of the product. Based on the measured temperatures of selected producers, we can describe the traditional smoking of fish in Cambodia as hot smoking with the temperature at the first tray level (50–100 cm from the base of the smoking kiln) in the smoking kiln ranging from 80 to 100 °C. A study by Han et al. (2020) focusing on the

influence of the combustion temperature and fuel type on PAHs emissions reported that temperature was the most important factor in PAHs formation. According to a study by Hokkanen et al. (2018), lower amounts of PAHs were formed when the temperature was optimized than when it was not optimized, i.e., it might vary during the process, as in our case. The optimized temperature in a later study was kept between 400–600 °C. Further temperature measurements focused on both the combustion temperature and temperatures of the smoke and product throughout the whole process are recommended to gather more robust data to evaluate the effect of temperature in this area.

3.3. Fish fat content and its association with PAHs concentration

It was already stated that the fat content of fish might affect the final PAHs content. Table 6 shows the mean fat content (in %) for each sampled fish species. The sample of *Paralaubuca typus* had the highest mean value (50.9 %), followed by *Clarias batrachus* (47.3 %) and *Osteochilus schlegeli* (40.7 %).

As shown in Table 6, the fat content varied greatly within each species. This result might be attributed to different smoking processes, lengths of the process, and ages and sizes of the fish. To date, there are no reports summarizing the dependence of the concentration of PAHs on the fish species commonly consumed in Cambodia or Southeast Asian countries or on their fat content. However, various authors have discussed the correlation between fat and the concentration level of PAHs in smoked fish products, particularly in Europe (Basak et al., 2010; Duedahl-Olesen et al., 2010).

The correlation between the fat content and PAHs concentration was evaluated. The results did not show a statistically significant correlation between the fat and PAHs contents. This trend could be explained by the extended length of the smoking process and consequent loss of fat. Therefore, we can assume that the level of contamination by PAHs is not affected by the fat content of the fish, but it is affected by a combination of other factors, as described above.

Table 6
Descriptive analysis of fat content of all species in % dry weight.

Fish	% fat (min)	% fat (max)	% Median fat	% Mean fat	% fat SD*
Belodontichthys truncatus	18.7	44.9	33.2	31.1	±9.9
Henicorhynchus siamensis	1.43	52.9	30.9	28.5	±14
Clarias batrachus	31.6	64.2	48.9	47.3	±8.1
Hypsibarbus malcolmi	37.2	43.7	39	40	±3.4
Labeo chrysophekadion	0.09	25.3	23.9	16.2	±12
Micronema hexapterus	0.09	46.7	21.8	22.9	±14
Notopterus notopterus	0.46	2.35	1.76	10.1	±1
Ompok bimaculatus	0.09	30.6	1.89	8.22	±12
Osteochilus schlegeli	37.6	46.7	37.7	40.7	±5.2
Pangasius elongatus	31.5	32.2	31.5	31.7	±0.4
Paralaubuca barroni	1.99	60.8	40.2	35.2	±26
Paralaubuca typus	26	80.2	45.1	51	±21
Phalacronotus bleekeri	8.26	23.7	10.7	12.9	±5.3
Phalacronotus micronemus	6.53	11.5	9.89	9.43	±1.8
Puntioplites prostozystron	30.6	44.1	37.8	37.6	±4.4
Rasbora hobelmani	16.7	61	31.2	34.6	±12
Wallago attu	4.99	8.02	6.34	6.45	±1.3
Xenentodon cancila	9.71	10.6	10.4	10.2	±0.5

* SD = standard deviation.

4. Conclusions

In conclusion, this study reported the levels of BaP, ΣPAH_4 and ΣPAH_{12} in 18 species of smoked fish commonly consumed in Cambodia for the first time. Overall, the results presented in this study highly exceeded the recommended levels of BaP and ΣPAH_4 according to the European Commission. The highest PAHs concentrations were detected in *Paralaubuca typus* fish sample (2700 μg/kg and 16,800 μg/kg) (P7), followed by *Labeo chrysophekadion* (P2) (3780 μg/kg and 13,500 μg/kg). The lowest mean values of ΣPAH_4 and ΣPAH_{12} measured in *Paralaubuca barroni* were 76.3 μg/kg and 537 μg/kg (P2), respectively. It was noted that consuming fish without the skin might decrease the level of carcinogenic PAHs ingested as well as the effects of direct sources of heat and substantial fat drippings into fire on the level of PAHs contamination. Regarding the fish species, the highest mean values of ΣPAH_4 and ΣPAH_{12} were measured for samples of *Paralaubuca typus* (1640 μg/kg and 9170 μg/kg, respectively). The age, season, and sample size can affect the PAHs content. The results showed that the mean values of ΣPAH_4 and BaP were significantly different ($p < 0.05$) at smoking times T1 (3–16 h) and T2 (1–4 days) in the cases of *Henicorhynchus siamensis, Micronema hexapterus, Paralaubuca barroni* and *Paralaubuca typus*. These results suggested that the concentrations of PAHs stabilized with time. Within a species, statistically significant differences ($p < 0.05$) in the PAHs concentration were observed between the types of fuel. The use of inappropriate fire starters such as plastics or gas was noted. Finally, the total fat content was measured, and the correlation between the fat content and PAHs contamination was analysed. A statistically significant correlation ($p < 0.05$) between the fat content and PAHs concentration was not proven. Further evaluation of the effects of the distance from the heat source and the measured temperatures, particularly the combustion temperature and temperatures of the smoke and product, through the whole process is recommended. The extremely high concentrations of PAHs measured in this study are attributed to a combination of factors, such as the type of fuel used, use of inappropriate fire-starting techniques, length of the process, use of a direct heat source and lack of temperature regulation systems, and they are discussed in detail. However, by following good manufacturing practices, PAHs contamination in smoked meat products can be controlled and decreased, maintaining the beneficial effects of smoking and preventing its undesirable effects.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

Not applicable.

CRediT authorship contribution statement

Tereza Slámová: Formal analysis, Methodology, Validation, Writing - original draft. **Adéla Fraňková:** Methodology, Data curation, Validation, Writing - original draft. **Jan Banout:** Supervision, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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